Structure

In This Issue

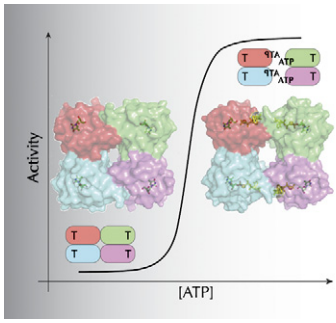
In this issue of *Structure*, we continue our Opinion series, with an aim to facilitate the ongoing debate about structural genomics, and with special focus on the National Institutes of Health (NIH)-funded Protein Structure Initiative. More details can be found in our Editorial published in November issue of *Structure*.

Feast, Famine, and Transcriptional Regulation under Extreme Conditions

PAGE 1542

In bacteria and archaea, nutrient levels have a dramatic impact on global regulation of gene expression, i.e., feast/famine regulation. The hyperthermophilic archaeon *Pyrococcus* sp. OT3 grows in hydrothermal vents, utilizing amino acids from polypeptidic debris as the food source. Yokoyama et al. now investigate structure and function of FL11, a transcription factor responsive to lysine levels and an example of a feast/famine regulator. Amazingly, FL11 is found to regulate transcription of ~200 genes involved in a variety of metabolic pathways. FL11 achieves this task by shifting dimer/octamer equilibrium, which governs FL11 affinity for promoter DNA.

Regulation of Thymidine Kinase 1-like Enzymes – It Takes Four to Tango



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The first phosphorylation step in thymidine triphosphate (TTP) synthesis salvage pathway is catalyzed by thymidine kinases (TKs). TKs are critical for controlling intracellular TTP levels and therefore subject to strict control themselves; for example, mammalian TK1 is expressed in the cytoplasm during the S-phase of the cell cycle and degraded by the proteasome after the exit from mitosis. This study by Segura-Peña et al. presents the first collection of high-resolution structures of a single member of the TK1-like enzyme family in different stages along the reaction coordinate. The authors provide evidence for the existence of two distinct quaternary structures in TK1: open (active) and closed (inactive). They suggest that transition between the two conformations is likely involved in regulating enzyme activity. (Figure credits Segura-Peña et al.)

Improved Protein Stability Estimation by Modeling Backbone Flexibility

PAGE 1567

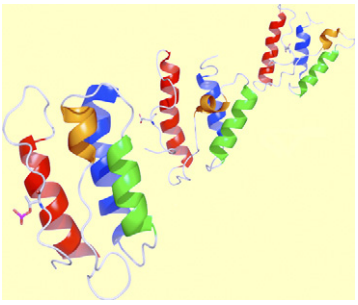
Creating specific mutations in a given protein is a widely used strategy employed to investigate details of protein structure and function. Here, Yin et al. present Eris, an automated estimator of mutation-induced protein stability changes. The performance was tested extensively and significant correlations between the predicted and experimental stability changes were found. Unlike previous methods, Eris effectively detects and resolves the atomic clashes and structure strains introduced by the mutations and yields reliable predictions. It also models the backbone flexibility, thereby allowing for determination of the mutation-induced backbone conformational changes. (Eris is freely accessible through http://eris.dokhlab.org.)

Bringing dRIFTing Protein Folds Closer

PAGE 1577

Bacterial and eukaryotic riboflavin kinases transfer a phosphate group from ATP to riboflavin to form flavin mononucleotide and are among several flavin-binding enzymes that share the RIFT β-barrel fold. Ammelburg et al. show that corresponding enzymes from archaea also have the RIFT barrel fold but are unique in using CTP as the phosphate source. Moreover, they show CTP-dependent riboflavin kinases to be evolutionary intermediates, linking ancient DNA-binding RIFT barrel proteins to modern enzymes.

Global Conformational Change behind Smooth Muscle Contraction



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Agonist-evoked smooth muscle contraction requires inhibition of myosin phosphatase by a phosphorylation-dependent inhibitor protein, CPI-17. CPI-17 is phosphorylated by protein kinase C (PKC) and Rho-associated kinase (ROCK) in response to G-protein activation and dephosphorylated during NO-induced relaxation. Therefore, CPI-17 is one of the critical components in smooth muscle contraction regulation. In this issue, Eto et al. report a solution structure of phosho-CPI-17 which reveals a global conformational change of CPI-17 triggered by phosphorylation. The authors conclude that CPI-17 conformational change is responsible for potent and specific inhibition of myosin phosphatase. (Figure credits Eto et al.)

Calmodulin Orchestrates N-Methyl-D-Aspartate Receptor

PAGE 1603

N-methyl-D-aspartate receptor (NMDAR) is a regulator of neuronal calcium flux and plays critical roles in learning, memory, neural development, and synaptic plasticity. NMDAR activity is regulated by Ca^{2+} levels through calmodulin (CaM) binding. Upon Ca^{2+} influx, CaM binds tightly to the C0 and C1 regions of the NMDAR NR1 subunit. Akyol Ataman et al. now present a crystal structure of Ca^{2+} loaded CaM in complex with a peptide corresponding to the C1 region of NMDAR NR1. The structure of this complex reveals that NR1 Ser890, whose phosphorylation regulates membrane localization, is solvent-protected, while the ER retention motif is solvent-exposed. Thus, CaM binding may play a role in control of NMDAR subcellular localization and trafficking.

In This Issue

Ras Residue 61 Conundrum

PAGE 1618

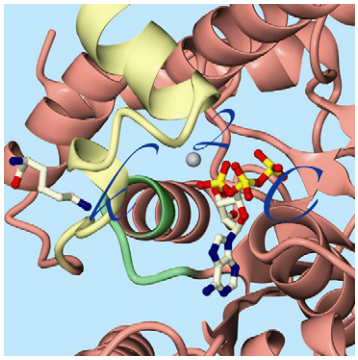
Mutations at residue 61 of Ras, a small monomeric GTPase, convert Ras to oncogenes characterized by a wide range of transforming efficiency. Curiously, although all mutant proteins display a decrease in the in vitro Ras GTPase activity equally, their transformation efficiencies in vivo vary over a 1000-fold range. Buhrman et al. now show that conformation of residue 61 is associated with transformation efficiency and that this efficiency is directly correlated with mutants' ability to stabilize a noncatalytic conformation in the context of the Ras/Raf complex. Thus, absence of Raf in the in vitro studies might be the key to understanding the observed Ras conundrum.

Structure Refinement at Low Resolution

PAGE 1630

Many experiments on biomolecules yield only low-resolution or sparse structural data. In those cases, the refinement of an initial model at low resolution can lead to severe overfitting and, eventually, incorrect structures. Schröder et al. developed an approach that employs a deformable elastic network (DEN) to combine prior structural knowledge with experimental data, preserving structural information that is not provided by the data. The DEN restraints dramatically reduce overfitting, especially at low resolution. The method is implemented in the program DireX, which is available through the SimTK website (https://simtk.org/home/direx).

Mechanistic Details of Cytidine to Lysidine Conversion



PAGE 1642

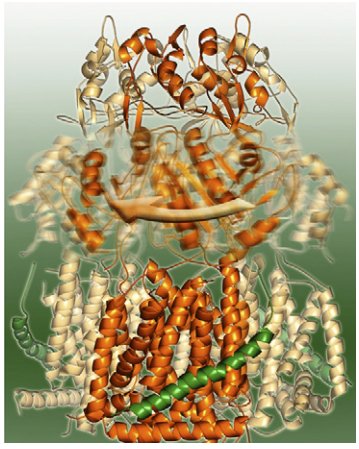
In a bacterial genetic-code system, decoding an AUA codon as isoleucine is achieved by the post-transcriptional modification of the cytidine residue of $tRNA^{Ile}_2$(CAU) to lysidine. This conversion is catalyzed by $tRNA^{Ile}$ lysidine synthetase (TilS) in two consecutive steps, utilizing ATP and L-lysine, respectively. Kuratani et al. report a series of TilS structures and show that TilS has two separate gateways: a large hole for ATP and a narrow tunnel for L-lysine. Initially, ATP, Mg^{2+}, and L-lysine are bound far apart. The authors propose that cytidine binding induces structural changes that bring ATP and Mg^{2+}closer and allow first transformation to occur. Next, another set of conformational changes brings L-lysine to vicinity of adenylated cytidine, leading to lysidine formation. (Figure credits Kuratani et al.)

"Rotation and Tilt" and "Hinge Bending" in KcsA Gating Mechanism

PAGE 1654

Potassium channels support permeation of K^+ ions across cell membranes. These channels continually fluctuate between closed and open states. Here, Miloshevsky and Jordan demonstrate the gating mechanism that opens the KcsA potassium channel and predict the open state conformation of KcsA in advance of a resolved structure. They show that the "rotation and tilt" and "hinge bending," proposed as mechanisms for gating, are not mutually exclusive. Rather, they describe different stages in the process of attaining the open state configuration. Thus, the transition is initiated by rotation and tilt; the bent hinge describes the channel's final open state.

Multidrug Efflux Pumps with a New Twist



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One of the ways bacteria avoid drug-induced damage is to use multidrug efflux pumps to export a broad variety of foreign chemical agents out of the bacterial cell before lethal concentrations are reached. In *Escherichia coli* the AcrB:AcrA:TolC efflux complex forms a principal transporter. Here, Törnroth-Horsefield et al. present the crystal structure of AcrB in complex with a new transmembrane protein, YajC. The structure revealed a specific rotation of the periplasmic porter domain of AcrB, consistent with the hypothesized mechanism for TolC opening. Although a functional role of the YajC:AcrB interaction could not conclusively be assigned, YajC deletion produces more a drug-susceptible phenotype. (Figure credits Törnroth-Horsefield et al.)

PufX-Mediated Dimerization of Bacterial Photosynthetic Core Complex

PAGE 1674

Purple photosynthetic bacteria from *Rhodobacter* species are model organisms for investigating photosynthesis in bacteria. Their photosynthetic machinery contains a core complex composed of the reaction center, light-harvesting complex, and the PufX protein. Here, Busselez et al. report a 3D cryo-EM reconstruction of the core complex of *Rba. veldkampii* that allowed them to visualize subunit organization and define the position of PufX. Their results suggest that dimerization of the core complexes is mediated by the transmembrane domains of PufX.